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Dated: _____

Docket No.: 03818/100L650-US1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Mladen Mercep et al.

Application No.: 10/615,716

Filed: July 8, 2003

Art Unit: 1623

For: COMPOUNDS, COMPOSITIONS AND
METHODS FOR TREATMENT OF
INFLAMMATORY DISEASES AND
CONDITIONS

Examiner: E. Peselev

DECLARATION OF LINDA TOMAŠKOVIĆ UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Linda Tomašković, declare that:

- (1) I am a citizen of Croatia and reside in Zagreb, Croatia.
- (2) I am currently employed at Pliva-Istrazivacki Institut d.o.o.
- (3) I am one of the named inventors of the above-identified application published as U.S. Pat. Pub. No. 2004/0014685
- (4) I re-affirm my duty of candor and good faith in dealing with the United States Patent and Trademark Office ("USPTO"), including the duty to disclose to the USPTO all information known to be material to the patentability of the invention as defined in 37 CFR §1.56.

{W:\03818\100L650US1\00612883.DOC *03818100L650US1* }

BEST AVAILABLE COPY

(5) I submit this declaration to support patentability of claims of the present application that have been rejected based on Burnet (US 2004/0087517) on the grounds of either anticipation (prior identical disclosure of the invention) or obviousness.

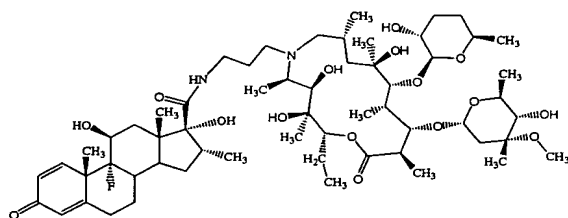
(6) More specifically, I submit this declaration to show that my co-inventors and I conceived and reduced the present invention to practice at a time prior to February 15, 2002, the earliest filing date asserted by Burnet.

(7) From a time considerably earlier than February 15, 2002, my co-inventors and I formed part of a team that devoted considerable resources to an on-going program of development of macrolide conjugates that include an anti-inflammatory moiety linked to a macrolide molecule and that possess anti-inflammatory activity. The macrolides were predominantly azithromycin and other homoerythromycin derivatives.

(8) As evidence of the existence and purpose of this program, and the existence of a broad concept that led to the present invention, I attach a copy of the specification of International Patent Application WO 02/055531 (Exhibit A). Three of my present co-inventors and I are named as inventors in WO 02/055531. This application was filed January 3, 2002. In this document, we broadly state that conjugates having the general formula MLA have anti-inflammatory activity. M represents a macrolide subunit (Formulas M1, through M6), L is a linker linking M and A, and "A represents an anti-inflammatory subunit that can be steroid or nonsteroid." (Exhibit A, paragraph bridging pages 3 and 4, page 3 lines 12-13, pages 4 - 9 (macrolides), and pages 9 -12 (steroids)). Exhibit A also discloses a number of specific macrolide-steroid conjugates (Exhibit A, Table 1, pages 39-43) and their anti-inflammatory activity (Exhibit A, page 3 and pages 25 - 29.

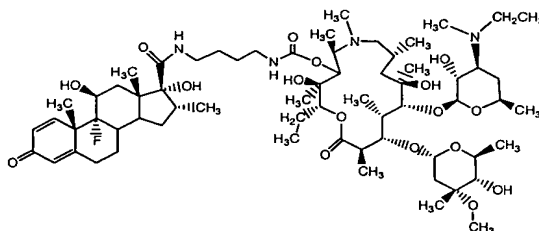
(9) Further, as evidence that my co-inventors and I reduced the present invention to practice prior to February 15, 2002 I submit pertinent excerpts from the laboratory notebook maintained in my laboratory pertaining to some of the compounds that were synthesized early. These entries were made prior to February 15, 2002.

(10) To show that, for example, Compound 1 of the present application was synthesized before February 15, 2002, I submit as Exhibit B pages 16-17, from Laboratory Notebook identified by code LTO0959016A Lab.diary 000959, and an English translation of these pages. Exhibit B sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 1 in the present application:



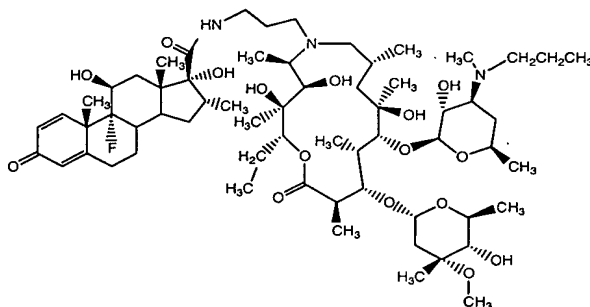
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also set forth on pages 16 - 17. Additionally, the synthesis of the macrolide-linker and the dexamethasone derivative used to form the conjugate compound 1 described above are provided with amount and identity of reagents used, solvents, and reaction yield in Exhibit C (pages 106-7 of Lab diary 0560, pages 98-99 of Lab diary 0560, and page 59 of Lab diary 0560). These records demonstrate that Compound 1 disclosed in the present application was actually reduced to practice prior to February 15, 2002.

(11) To show that, for example, Compound 4 of the present application was synthesized before February 15, 2002, I submit as Exhibit D pages 77-78 from a Laboratory Notebook identified by code VPO-1131- 077-A Lab.diary 1131, and an English translation of these pages. Exhibit D sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 4 in the present application:



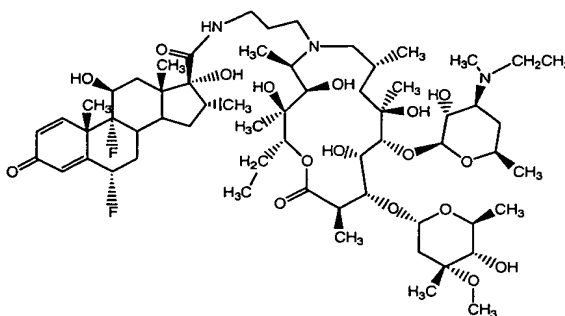
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 4 within the present application was actually reduced to practice prior to February 15, 2002.

(12) To show that, for example, Compound 9 of the present application was synthesized before February 15, 2002, I submit as Exhibit E page 124 from a Laboratory Notebook identified by code OMA-000961-124-A Lab.diary 000961 and page 22 OMA-001127-022-A Lab.diary 001127, and an English translation of these pages. Exhibit E sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 9 in the present application:



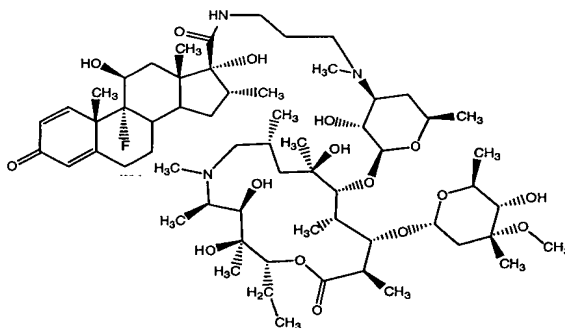
The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. Compound 9 was prepared using the procedure according to the procedure for compound 19, discussed below. This record demonstrates that Compound 9 within the present application was actually reduced to practice prior to February 15, 2002.

(13) To show that, for example, Compound 19 of the present application was synthesized before February 15, 2002, I submit as Exhibit F pages 118-119 from a Laboratory Notebook identified by code OMA-000961-118-A Lab.diary 000961, and an English translation of these pages. Exhibit F sets forth the synthetic protocol for the conjugation of the macrolide and flumethasone which produced the conjugate designated as Compound 19 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 19 within the present application was actually reduced to practice prior to February 15, 2002.

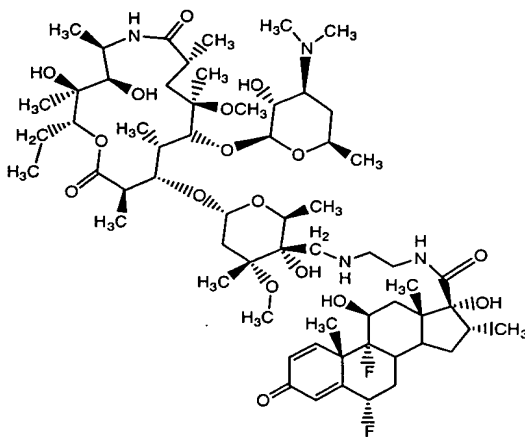
(14) To show that, for example, Compound 23 of the present application was synthesized before February 15, 2002, I submit as Exhibit G page 5 from a Laboratory Notebook identified by code OMA-1127-005-A Lab.diary 1127, and an English translation of this page. Exhibit G sets forth the synthetic protocol for the conjugation of the macrolide and dexamethasone which produced the conjugate designated as Compound 23 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. This record demonstrates that Compound 23 within the present application was actually reduced to practice prior to February 15, 2002.

(15) To show that, for example, Compound 27 of the present application was synthesized before February 15, 2002, I submit as Exhibit H pages 48 - 49 from a Laboratory

Notebook identified by code VPO-1131- 048-A Lab.diary 1131 and an English translation of these pages. Exhibit H sets forth the synthetic protocol for the conjugation of the macrolide and flumethasone which produced the conjugate designated as Compound 27 in the present application:



The amount and identity of reagents used, solvents, yield, and product analytical data (MS, and purity) are also provided. Compound 27 was prepared by the process provided in pages 20 – 21 of the Laboratory Notebook identified by code VPO-1131- 020-A Lab.diary 1131, submitted as Exhibit I. These records demonstrate that Compound 27 within the present application was actually reduced to practice prior to February 15, 2002.

(16) My laboratory worked diligently on conception and diligent reduction to practice of the claimed invention from a time prior to February 15, 2002, until the filing of provisional application 60/394,670 on July 8, 2002 and thereafter until the filing date of the present application on July 8, 2003. For example, the provisional application 60/394,670 discloses techniques for synthesis of 33 compounds, including Compounds 1, 4, 9, 19, 23, and 27 discussed above, as well as 27 additional compounds that were synthesized prior to filing the provisional application. At least 7 further compounds were synthesized prior to filing the non-provisional application. All of these 40 compounds were synthesized between the conception date (prior to February 15, 2002) and the filing date of the present application. This demonstrates that we worked diligently in order to reduce the claimed compounds to practice.

(17) I declare further that statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 29.12.2005.


Linda Tomašković

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIĆ

№ 000959

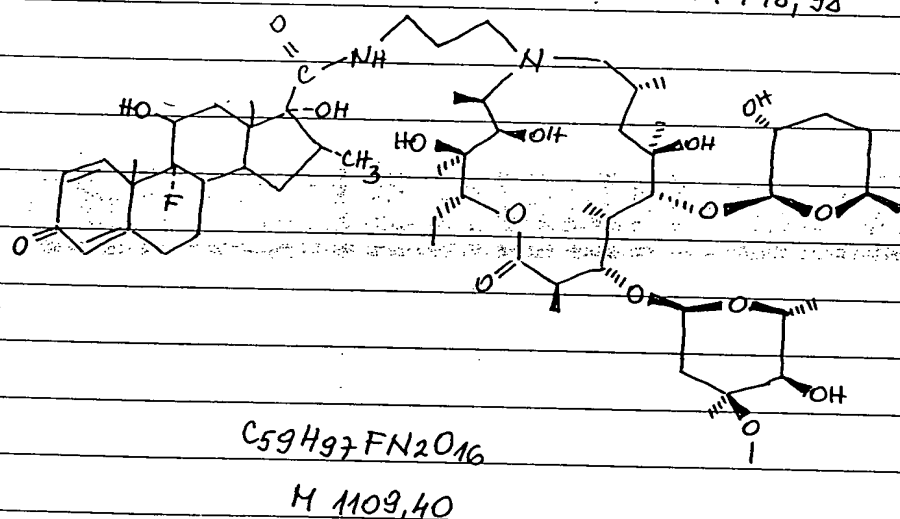
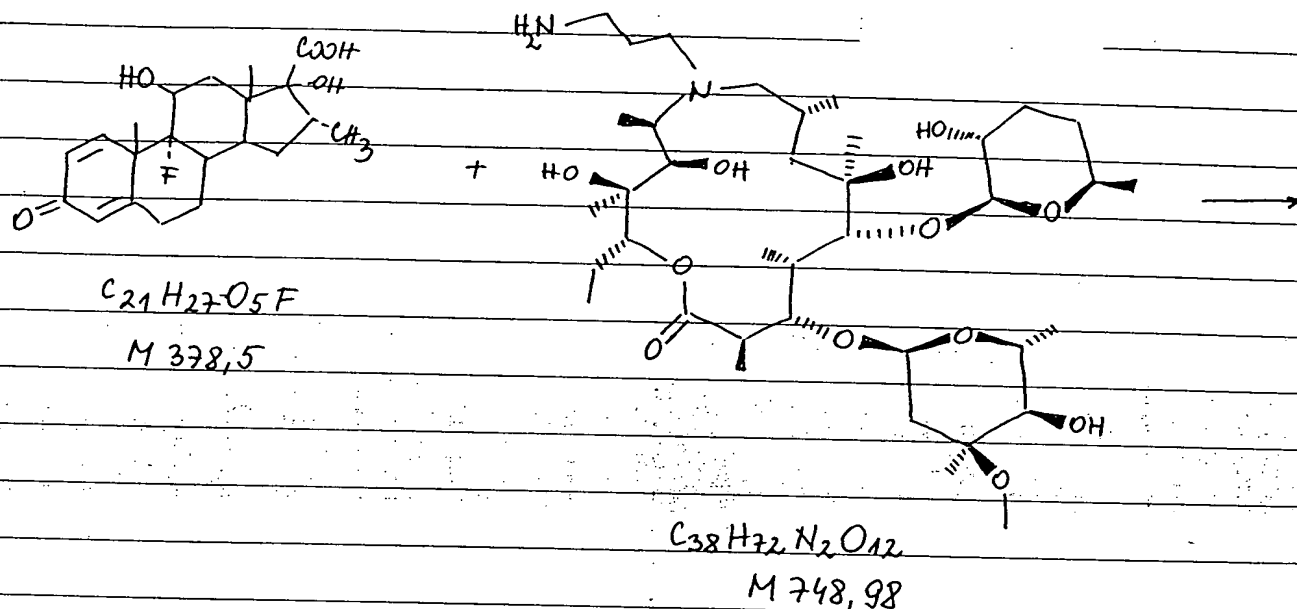
Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



Naslov: _____

Oznaka LT 77

Datum (i): 30. 04. 2001.



ref: D. Ramo et. al., J. Am. Chem. Soc. 120 (1998) 12 237

KEMIKALIJE:

1-hydroxybenzotriazole, ACROS, M 135, 12

1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide · HCl, ALDRICH,
M 191,71

Rad obavio: Linde Tomasić

Datum: 30. 04. 2001.

Rad posvjedočio: Dr. M. B. B. B.

Datum: 30. 4. 2001.

Naslov: _____

Oznaka LT 77

Datum (i): 30.04.2001.

Postupak je opisan u dnevniku 0560, M. 106.

Količine reaktanata:

debrametaxon-hidroklorid 76 mg ; 0,2 mmol

TEA 0,253 ml

HOBt 53 mg

amin 150 mg ; 0,2 mmol

EDC · HCl 157 mg

Dohvaćeno je 0,1666 g čistog proizvoda. Koristi se
reakcije 75%.

MS(ES⁺) : 1109,7 (MH⁺)

MS(ES⁻) : 1107,9 (MH⁻)

Rad obavio:

Andre Tomastunec

Datum:

30.04.2001.

Rad posvjedočio:

Andre Tomastunec

Datum:

30.4.2001.

Title: _____

LabDiary ID

LT77
PL14882

Date (i): April 30, 2001

Synthetic scheme: Lab. Diary No. 000959, page 16

Chemicals:

1-hydroxybenzotriazole, ACROS, M 135.12

1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, ALDRICH, M 191.71

Performed by: Linda Tomašković

Date: April 30, 2001

Witnessed by: Ivana Ozimec

Date: April 30, 2001

Title: _____

LabDiary ID LT77

Date (i): April 30, 2001

Reaction procedure and data about other chemicals which are used is described in Laboratory Diary Number 0560, page 106.

Amounts:

dexamethasone acid	76 mg; 0.2 mmol
TEA	0.253 ml
HOBT	53 mg
amine	150 mg; 0.2 mmol
EDC·HCl	157 mg

0.1666 g of pure product was obtained. Reaction yield 75%.

MS(ES^+) : 1109.7 (MH^+)

MS(ES^-) : 1107.9 (MH^-)

Performed by: Linda Tomašković
Witnessed by: Ivana Ozimec

Date: April 30, 2001
Date: April 30, 2001

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIĆ

№ 000560

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

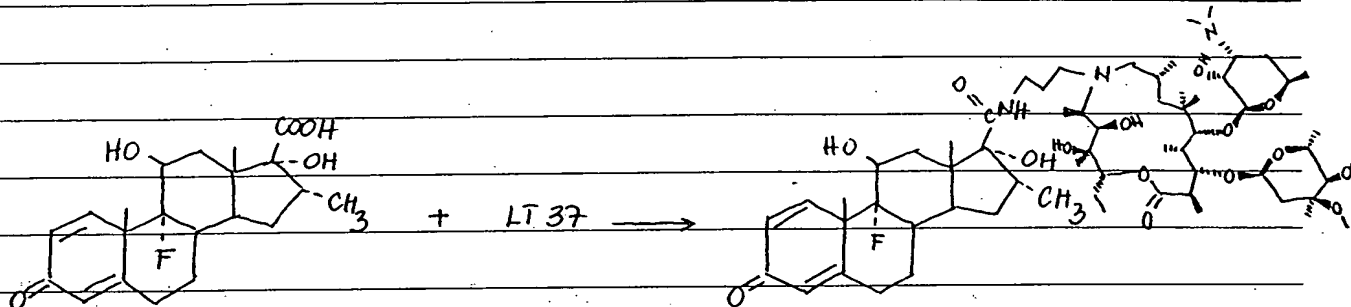


Naslov: _____

Oznaka LT40

Datum (i): 23.02.2000

PLR 2146



$C_{21}H_{27}O_5F$

M 378,50

$C_{40}H_{77}O_{12}N_3$

M 792,4

$C_{61}H_{102}FN_3O_{16}$

M 1152,47

ref: D. Romo et al., J. Am. Chem. Soc. 120 (1998) 12237

KEMIKALE:

1-HYDROXYBENZOTRIAZOLE, ACROS, M 135,12

1-(3-DIMETHYLAMINOPROPYL)-3-ETHYL-CARBODIIMIDE HYDROCHLORIDE,
ALDRICH, 28747-069; M 191,71

U suspenziju hisklina LT4 (110 mg; 0,29 mmol) u suhom dihlormetanu (5 ml) ohlađenom na 0°C dodamo je 0,380 ml trietilamina (otopina u razbistrila), zatim HOBt (80 mg; 0,59 mmol), amin LT37 (230 mg; 0,29 mmol) i 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (235 mg; 1,2 mmol). Reakcija je provedena na sobnoj temperaturi 24 h uz argon i miješanje.

Rad obavio:

Linda Tomasić

Datum:

23.02.2000

Rad posvedočio:

Andrija Anđelić

Datum:

13.03.2000

Naslov: _____

Oznaka LT40

Datum (i): 24. 02 2000

Nakon 24 h reakcija mješera je uparena na manji volumen na rotarapotu (~ 2 ml) i očišćena na koloni punjenoj sa silikagelom (eluens: CHCl_3 : MeOH: NH_4OH = 6:1:0,1). R_f produkta = 0,57. Dobiveno je 224 mg čistog produkta (ishodnostnje reakcije 67%).
MS (ES⁺): 1152,2 (MH⁺)

Rad obavio: Andre Tomasić

Datum: 24. 02 2000

Rad posvjedočio: Antun Šturm

Datum: 13. 03. 2000

Title: _____

LabDiaryID

LT40
PLR 2146

Date (i): February 23, 2000

Synthetic scheme: Lab. Diary No. 000560, page 106

Chemicals:

1-hydroxybenzotriazole, ACROS, M 135.12

1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, ALDRICH, 28747-069; M 191.71

To a suspension of acid LT4 (110 mg; 0.29 mmol) in dry dichloromethane (5 mL) cooled to 0°C under argon, 0.380 mL of triethylamine was added (the solution was cleared), then HOBT (80 mg; 0.59 mmol), amine LT37 (230 mg; 0.29 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (235 mg; 1.2 mmol) were added. The reaction mixture was stirred for 24 hours at room temperature in a flow of argon.

Performed by: Linda Tomašković
Witnessed by: Milan Mesić

Date: February 23, 2000
Date: March 13, 2000

Title: _____

LabDiaryID

LT40

Date (i): February 24, 2000

After 24 hours the reaction mixture was evaporated to a smaller volume on a rotary evaporator and purified on a silica gel column (eluant: CHCl_3 : MeOH : NH_4OH = 6 : 1 : 0.1). R_f of the product = 0.57. 224 mg of pure product was obtained (yield obtained 67%)
MS(ES^+) : 1152.8 (MH^+)

Performed by: Linda Tomašković
Witnessed by: Milan Mesić

Date: February 24, 2000
Date: March 13, 2000

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIC

№ 000560

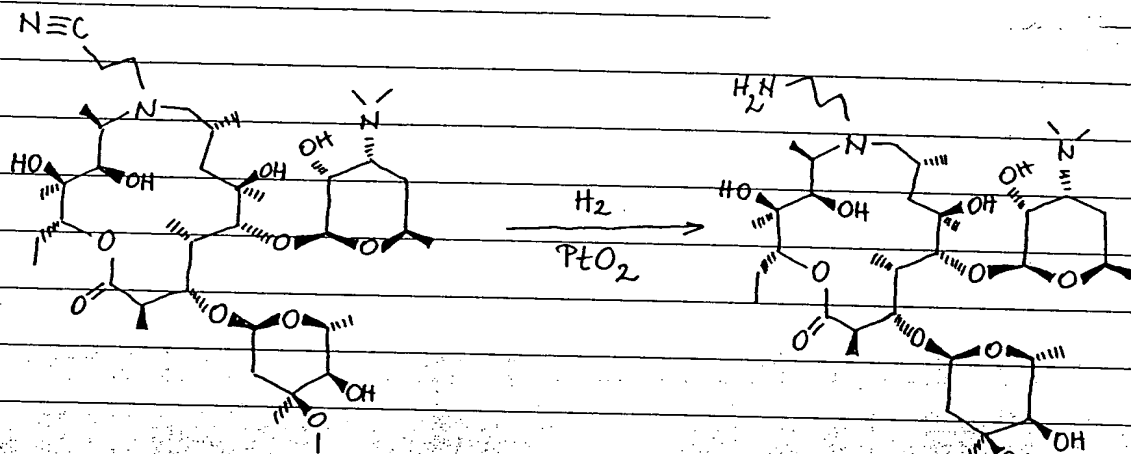
Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



Naslov: _____

Oznaka LT 37

Datum (i): 11. 02. 2000



LT35; $\text{C}_{40}\text{H}_{73}\text{O}_{12}\text{N}_3$ (M 788,4)

$\text{C}_{40}\text{H}_{77}\text{O}_{12}\text{N}_3$ (M 792,4)

ref: D. G. Batt et al., J. Med. Chem. 43 (2000) 41.

KEMIKALIJE:

Platinum(IV) oxide, M 227,09, laboratorij 11

LT 35 (0,7398 g; 0,94 mmol) otopljen je u 40 ml etanola i u to je dodana 130 mg PtO_2 . Reakcijska smjesa je stavljena u autoklav i reakcija je provedena uz miješanje i hidriranje pri tlaku od 30 atm 19 h. Nakon toga reakcijska smjesa je profiltrirana i etanol je upoten na rotarapori. TLC (eluent: CHCl_3 : MeOH: NH_4OH = 6:1:0,1) pokazuje smjesu polarnog smjesa ($R_f = 0,58$) i produkta ($R_f = 0,13$).

Rad obavio: ginka Tomasić

Datum: 11. 02. 2000

Rad posvjedočio: Milica Pril

Datum: 18. 7. 2000.

Naslov: _____

Oznaka LT 37

Datum (i): 12.02.2000

Reakcijska smjesa je očišćena na koloni pripremljenoj
za silikagelom (eluens: CH_2Cl_2 : MeOH : NH_4OH = 30 : 50 : 2)
Doliveno je 108 mg čistog produkta. Yshovistenje
reduje 15%. MS (ES^+): 792,6 (MH^+)

Rad obavio: Linde Tomazović

Datum: 12.02.2000

Rad posvjedočio: Ljiljana Puc

Datum: 19.7.2000.

Title: _____

LabDiaryID

LT37
PLR 2150

Date (i): February 11, 2000

Synthetic scheme: Lab. Diary No. 000560, page 98

Chemicals:

Platinum(IV)oxide, M 227.09, laboratory 11

LT35 (0.7398 g; 0.94 mmol) was dissolved in 40 ml ethanol and 130 mg PtO₂ was added. The reaction was performed in an autoclave during 19 hours under the pressure of 30 atm. Subsequently, the reaction mixture was filtered and ethanol was evaporated on a rotary evaporator. TLC (eluant: CHCl₃ : methanol : NH₄OH = 6 : 1 : 0.1) showed mixture of starting compound (R_f = 0.58) and product (R_f = 0.13).

Performed by: Linda Tomašković
Witnessed by: Dijana Pešić

Date: February 11, 2000
Date: February 18, 2000

Title: _____

LabDiaryID

LT37

Date (i): February 12, 2000

Reaction mixture was purified on a silica gel column (eluant: CH_2Cl_2 : MeOH : NH_4OH = 30 : 50 : 2). 108 mg of pure product was obtained. Yield obtained 15%. MS(ES^+) : 792.6 (MH^+)

Performed by: Linda Tomašković
Witnessed by: Dijana Pešić

Date: February 12, 2000
Date: February 18, 2000

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

LINDA TOMAŠKOVIĆ

№ 000560

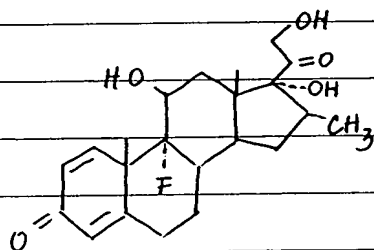
Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



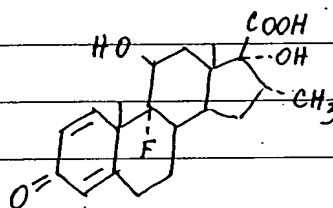
Naslov: _____

Oznaka: LT4

Datum (i): 20.04 '99.



M 392,47



M 378,5

ref: K. Meyer und J. Reichstein, Helv. Chim. Acta 30(1947) 1508

KEMIKALIJE

DEKSAMETAZON - PLIVA, šifra standarda 0033, knjiški broj 04

H₂O₂ - KEMIKA M192

DIOKSAN, p.a. - KEMIKA S: 9-16-33

U okrugloj tiknici od 100 ml otopljeno je 1g (2,5 mmol) deksametazona u 50 ml dioksana. U otopinu je zatim dodano 1,44 g (7,5 mmol) periodatne kiseline otopljene u 20 ml H₂O. Reakcija je provedena na sobnoj temperaturi 3h. Dioksan je uparjen na rotapoputu, a ostatak je razrijeđen s razjedajućom H₂SO₄. Bijeli kristalinični talog je ofiltriran preko Büchnera.

Dobiveno je 0,8642 g produkta (iskorištenje reakcije 90%).

Rad obavio: Đorđe Tomićević

Datum: 20.04 '99.

Rad posvjedočio: Milan Perić

Datum: 5.6.1999.

Title: _____

LabDiaryID

LT4

Date (i): April 20, 1999

Synthetic scheme: Lab. Diary No. 000560, page 59

Chemicals:

Dexamethasone- PLIVA, standard code 0033, serial number 04

HIO₄- KEMIKA M192

Dioxane, p.a.- KEMIKA s:9-16-33

Dexamethasone (1g; 2.5 mmol) was dissolved in 50 ml dioxane in a 100-ml round-bottomed flask. To this solution was added periodic acid (1.44 g; 7.5 mmol) dissolved in 20 ml H₂O. The reaction was performed at the room temperature for 3 hours. Dioxane was then evaporated on a rotary evaporator and the residue was acidified with diluted H₂SO₄. The white precipitate was filtered off by using Buchner funnel. 0.8642 g of product was obtained. Reaction yield obtained 90% .

Performed by: Linda Tomašković
Witnessed by: Dijana Parat

Date: April 20, 1999
Date: June 06, 1999

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

VIŠNJA POLJAK

1131

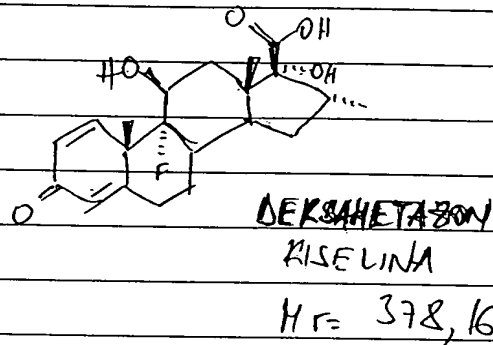
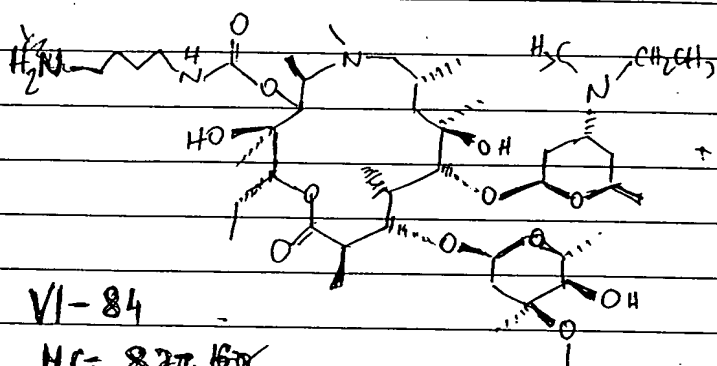
Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



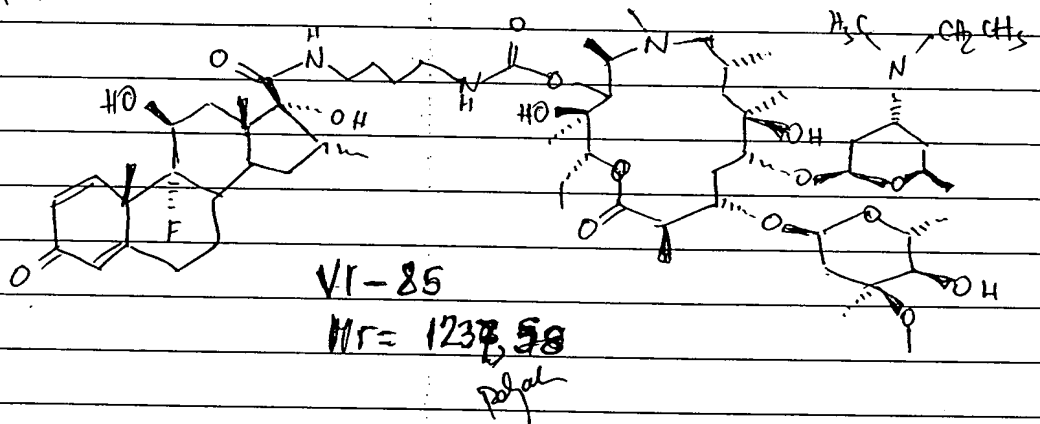
Istraživanje i razvoj

Naslov: _____ Oznaka VI-85
Pl 22714

Datum (i): 29. 1. 2002.



HOBt, TEA
EDC-HCl



Referenca:

POLAZNI MATERIJAL:

VI-84, Mr = 876,60

DEKSAHETAZON KISELINA, Mr = 378,16

TRIEILAMIN, 12NAD KOH

HIDROKSIBENZOTRIAZOL, ACROS lot-Nr: 1012582001

EDC-HCl, poruđen iz sobe 6

DIKLORMETAN, osušen nad molekulskom sita 0,3 mm

Rad obavio: Vijeta Poljak

Datum: 29. 1. 2002.

Rad posvjedočio: Linke Tanasinić

Datum: 29. 01. 2002.

Naslov: _____ Oznaka VI-85Datum (i): 29.1.2002.POSTUPAK DOZIVANJA:

U plinici propuhavoj argonom otopljeno je 423,0 mg (0,3332 mmol) dehidroclorid kiselike u 15 ml dihlormetana (suhog).
 U reakcijsku smjesu dodano je zatim 0,3632 ml (7,818 eg) tri-
 etilamina, pri čemu se otopina nazbisti. Dodano je zatim
 0,0900 g (2 eg) hidroksibenzoilazola, te brzo 292,1 mg
 (0,3332 mmol) otopja VI-84. U otopini je na kraju dodano
 još 4 eg vodušica 0,2272 g EDC-Hcl. Reakcijska smjesa je
 kućena na sobnoj temperaturi 24 h. Otapalo je upareno a
 spoj očisti na koloni u sustavu otapala $\text{CH}_2\text{Cl}_2 : \text{HOAc} : \text{NH}_4\text{OH} = 6:1:0,1$
 2. putom je očisti u sustavu $\text{CH}_2\text{Cl}_2 : \text{HOAc} : \text{NH}_4\text{OH} = 90:8:1$.

DOZIVENO PRODUKTA:

$$\text{AN (VI-85, oH)} = 0,0578 \text{ g}$$

Plasice pročišćene u kiležnici

$$\text{MS (NH)} = 1237,82$$

jelitni u: prologu.

Rad obavio: Olivera PoljakDatum: 29.1.2002.Rad posvjedočio: Linde TomatinićDatum: 29.01.2002.

PLIVA d.d.
Research Institute

77

Title: _____

Lab diary ID VI-85
 PL 27714

Date (i): 29.1. 2002.

Scheme in lab notebook No. 1131

Reference:

STARTING MATERIAL:

VI 84, Mr=876,60
Dexamethasone acid, Mr=378,16
triethylamine, over KOH
Hydroxybenzotriazole, Acros, Lot.NO. AO12582601
EDCxHCl, borrowed from the room 6
dichloromethane, dried over molecular sieves 0,3 nm

Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 29.1. 2002.

Date: 29.1. 2002.

Title: _____

Lab diary ID VI 85

Date (i): 29.1. 2002.

PREPARATION METHOD:

In a flask blown up with argon, 126,0 mg (0,3332 mg) of dexamethasone acid was dissolved in 15 ml of (dry) dichloromethane.

In the reaction mixture 0,3632 ml (7,818 eq) of triethylamine was then added, and the mixture cleared.

0,0900 g (2eq) of hydroxybenzotriazole was added, and then quickly 292,1 mg (0,3332 mmol) of compound VI-84. In the mixture was in the end added 4 eq i.e. 0,2292 g EDCxHCl. The reaction mixture was stirred at room temperature for 24 h. Solvent was then evaporated, and the product purified on a column in the solvent system $\text{CH}_3:\text{MeOH}:\text{NH}_4\text{OH}=90:8:1$.

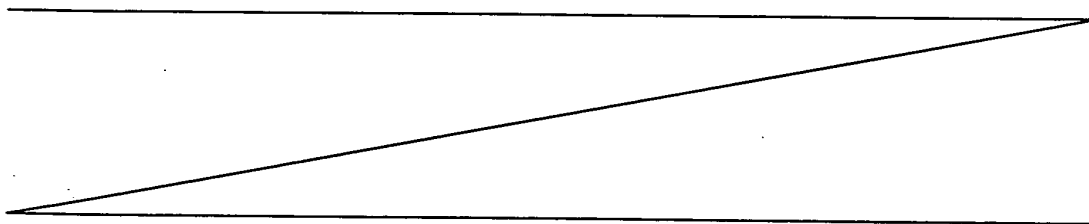
PRODUCT OBTAINED:

m(VI-85)= 0,0578 g

TLC plates in the notebook.

MS(MH+)=1237,82

Spectra in attachment.



Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 29.1. 2002.
Date: 29.1. 2002.



LABORATORIJSKI DNEVNIK

№ 000961

ORESTA MAKARUVA

DNEVNIK 2

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

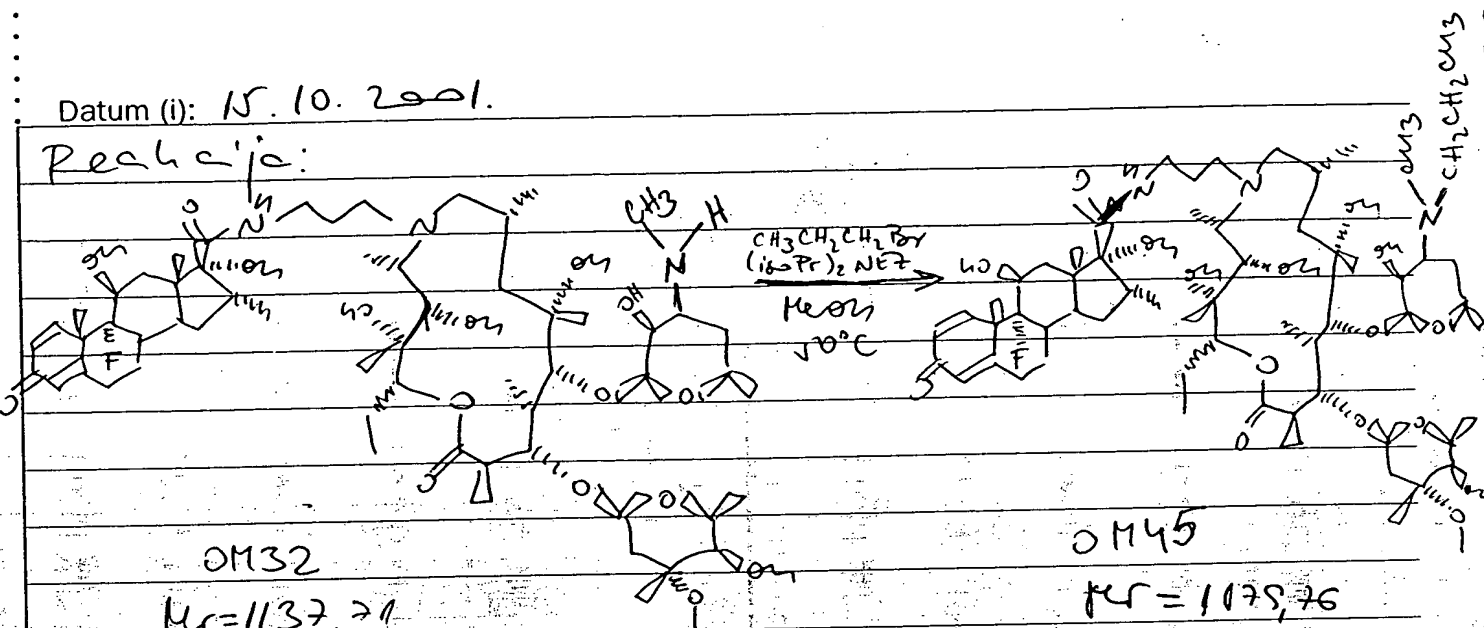


Naslov: _____

Oznaka OM45-1

Datum (i): 15. 10. 2001.

Reakcija:



$M_r = 1137,71$
 $C_{60}H_{100}FN_3O_{16}$

$M_r = 1178,76$
 $C_{63}H_{106}FN_3O_{16}$

Kemikalije:

OM32 $m = 90 \text{ mg}$ $m = 0,079 \text{ mmol}$ $\rho = 0,76 \text{ g/cm}^3$
 $(iBuPr)_2NEt$ $m = 0,6717 \text{ mmol}$ $V = 115 \mu\text{l}$ $M_r = 129,25$
 $CH_3CH_2CH_2Br$ $m = 0,1011 \text{ mmol}$ $M_r = 123$ $\rho = 1,35 \text{ g/cm}^3$ $V = 45 \mu\text{l}$
 $MeOH$ $V = 2 \text{ ml}$

Referencen i postupak opitni da su M_r 118/119.

Čistoća je na hladnom proučeni s IR i NMR
 u etereu $CHCl_3$: $MeOH$: NH_4OH = 6:1:0,1

$m(OM45) = 20 \text{ mg}$
 $m(OM45 \text{ met. amorf}) = 26 \text{ mg}$

MS (MH^+) 1180,59

Rad obavio:

Dr. sc. J. J. J.

Datum: 24. 10. 2001.

Rad posvjedočio:

Prof. dr. Tomislav

Datum: 24. 10. 2001.

Title: _____

LabDiaryID

OM45-1

Date (i): October 15, 2001

Synthetic scheme: Lab. Diary No. 000961, page 124

Chemicals:

OM32 m=90 mg n=0.079 mmol $\rho=0.76 \text{ g/cm}^3$

(isoPr)₂NEt n=0.6717 mmol V=115 μL M 129.25

CH₃CH₂CH₂Br n=0.5011 mmol M 123 $\rho=1.35 \text{ g/mL}$ V=45.7 μL

MeOH - V=2 mL

Reference and procedure are described on pages 118/119.

The reaction mixture was purified on a silica gel column with eluant: CHCl₃ : MeOH : NH₄OH = 6 : 1 : 0.1.

m(OM45)=20 mg

m(OM45 unpure)=26 mg

MS(MH⁺): 1180.59

Performed by: Oresta Makaruha

Witnessed by: Linda Tomašković

Date: October 24, 2001

Date: October 24, 2001

PLIVA d.d.

Istraživački institut

LABORATORIJSKI DNEVNIK

№ 000961

ORESTA MAKARUNA

DNEVNIK 2

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska

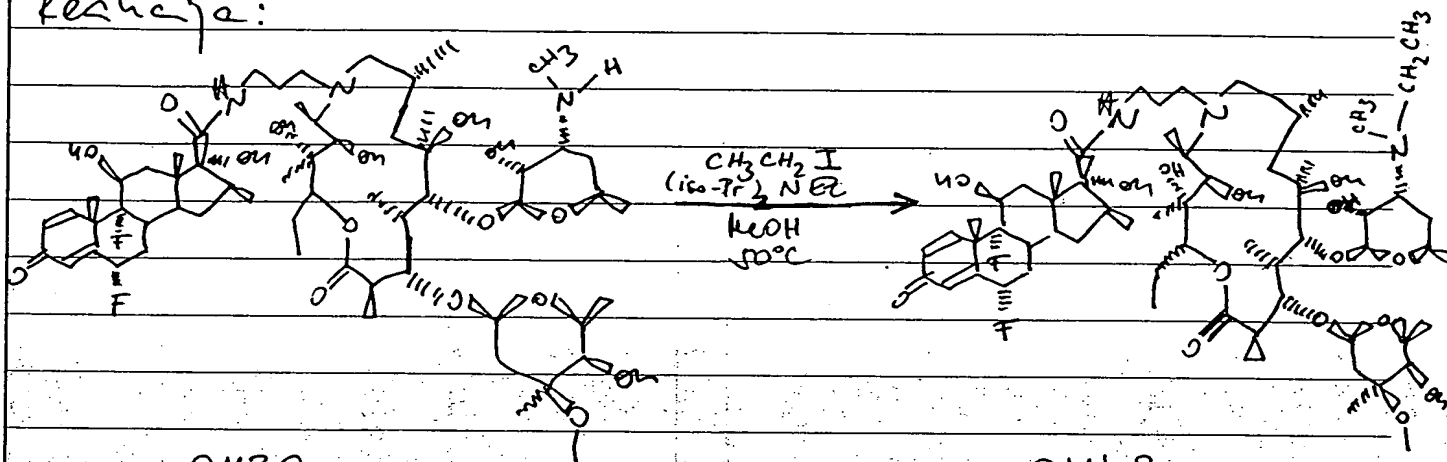


Naslov: _____

Oznaka OM43-1

Datum (i): 25.09.2001.

Reakcija:



OM39

$M_r = 1155,70$

$C_{60}H_{99}F_2N_3O_{16}$

OM43

$M_r = 1183,75$

$C_{62}H_{103}F_2N_3O_{16}$

Ref. K. Tanaka, T. Sano, S. Mami, H. Toyooka,
S. Omura, N. Inatomi, Z. Itoh; Chem. Pharm.
Bull. 37 (10) 2687-2700 (1989)

Kemijske:

OM39 ($M_r = 1155,70$) $n(OM39) = 0,0588 \text{ mmol}$ $n((\text{iso-Pr})_3\text{NEt}) = 0,5 \text{ mmol}$
N,N-diisopropylethylamine, Merck, 525410 844, $\rho = 0,72$
1-iodoethane, powder iz pte 6. $\rho = 1,910 - 1,940$ $M_r = 129,25$
MeOH, kemija $n(\text{CH}_3\text{CH}_2\text{I}) = 0,373$ $M_r = 155,97$

Postupak:

M. tjevnica od 10 ml otopine je 68 mg OM39 u
2 ml MeOH. Otopini je dodano 85,6 μL N,N-di-
isopropylethylamine i 112,5 μL iodoethane.
Mješavina je na magnetskoj mješalici 20 sati,
na temperaturi od 50°C. Reakcijska otopina

Rad obavio:

Dr. sc. [signature]

Datum: 26.09.2001.

Rad posvjedočio:

Simone Tomasić

Datum: 2.10.2001.

Naslov: _____

Oznaka OM33-1

Datum (i): 25.09.2001.

Prizjeto je s 20ml EtOAc i istraživač s 20ml NaClO_2 (zrnc.) i 20ml vno. Organička faza nakon je na bezvodnom Na_2SO_4 . Ostatak je uparen u rotacionom.

Sljedeći produkt i polaznog gvođa dišće je na laboratorijskim prilikama, a izračun na osnovu CHCl_3 : NaOH : Na_2SO_4 = 6:1:9,1.

$m(\text{OM43}) = 38 \text{ mg}$ dišće 95%

$m(\text{OM39 neizvježđeno}) = 24 \text{ mg}$

KS (K⁺) 1185.7

Rad obavio:

Orest Pribić

Datum:

26.09.2001.

Rad posvjedočio:

Finola Pribić

Datum:

2.10.2001.

Title: _____

LabDiary ID

OM43-1

Date (i): September 25, 2001

Reaction:

Synthetic scheme: Lab. Diary No. 000961, page 118

REF. : K. Tsuzuki, T. Sunazuka, S. Marni, H. Toyock, S. Omura, N. Inatomi, Z. Itok; Chem. Pharm. Bull. 37 (10) 2687-2700 (1989)

Chemicals:

OM39 (Mr=1155,70) n(OM39)=0,0588 mmol

N,N-diisopropylethylamine, Merck, S25410 844, $\delta=0,76$ kg/l; Mr=129,25; n((iso-Pr)₂NEt)=0,5 mmol

1-iodoethane borrowed from room 6. $\delta=1,910-1,940$ Mr=155,97 n(CH₃CH₂I)=0,373

MeOH, Kemika

Procedure:

In flask (10 ml) was dissolved 68 mg of compound OM 39 in 2 ml of MeOH. In the solution were added 85,6 μ l of N,N-diisopropylethylamine and 112,5 μ l of iodoethane. The reaction mixture was stirred at 50 °C for 20 hours. The reaction mixture

Performed by: Oresta Makaruha
Witnessed by: Linda Tomašković

Date: September 26, 2001
Date: October 02, 2001

Title: _____

LabDiary ID

OM43-1

Date (i): September 25, 2001

was diluted with 20 ml of EtOAc and washed with 20 ml of NaHCO₃ (satur.) and 20 ml of H₂O. Organic layer was dried over anhydrous Na₂SO₄. Solvent was evaporated under vacuum.

The mixture of product and initial compound was purified on a silica gel column in the solvent system CH₂Cl₂:MeOH:NH₄OH 6:1:0,1.

m (OM43)=89,1 mg purity 95%

m (OM39, initial compound)=24 mg

MS (MH⁺) 1185,7

Performed by: Oresta Makaruha
Witnessed by: Linda Tomašković

Date: September 26, 2001
Date: October 02, 2001

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

ORESTA MAKARUNA

DNEVNIK 3

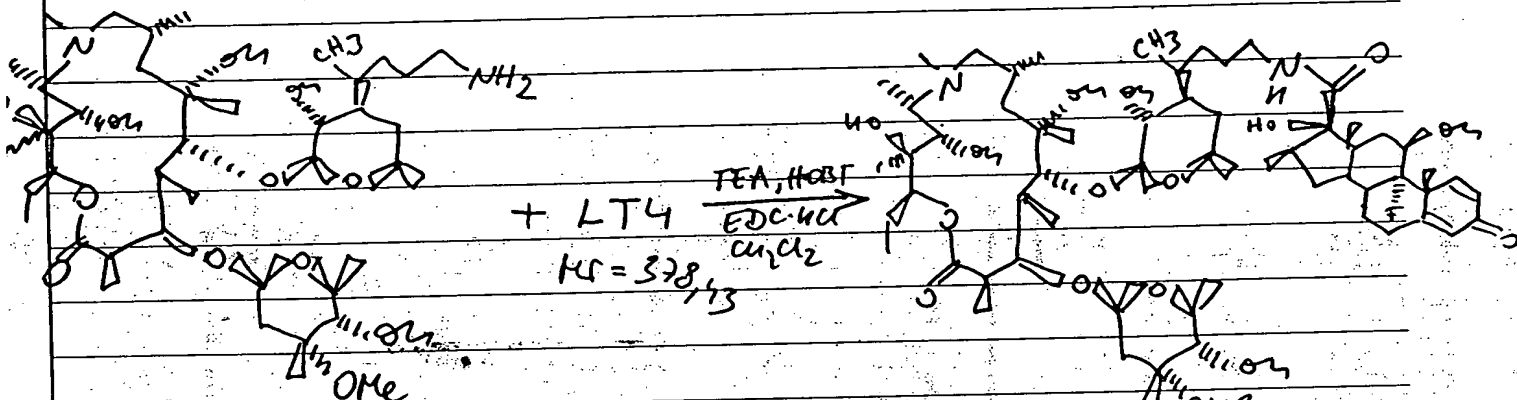
1127

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



Datum (i): 19. 11. 2001.

Reakcija:



OMX

MS = 791,55

OMY

MS = 1151,72

Referencija i postupak opisani su u PW. 56/57
u laboratorijском dnevniku br. 917!!

Kemijske količine:

OMX MS = 791,55 $m = 0,221 \text{ g}$ $m = 175 \text{ mg}$

CH_2Cl_2 $V = 5 \text{ mL}$

LT4 (dobiven od L. Tomatić, lab. dn. br. 506, PW. 98) $m = 9,221 \text{ g}$
 $m = 83,74 \text{ g}$

HOBT $m = 23 \text{ g}$ 61 mg

EDC·HCl $m = 179,22 \text{ mg}$

TEA $V = 290 \mu\text{L}$

Prodotak je čist i bez boje, dobiven u količini 15,8 mg.
Kupelom uz element CHCl₃:MeOH:NH₄OH = 6:1:0,1.

$m(\text{OMY}) = 15,8 \text{ mg}$ $95,11\% \text{ pri}$ $MS(MH^+) 1152,53$

Rad obavio:

Datum: 20. 11. 2001.

Rad posvjedočio:

Datum: 27. 11. 2001.

Title: _____

LabDiary ID

OMY-1
PL27439

Date (i): November 19, 2001

Reaction:

Synthetic scheme: Lab. Diary No. 001127, page 5

Reference and reaction procedure are described in Laboratory Diary Number 0917, pages 56/57

Chemicals:

OMX Mr=791,55 n=0,221 mmol m=175 mg

CH₂Cl₂ 5 ml

LT4 (received from Linda Tomašković, Lab. Diary No. 000506, page 98) n=0,221 mmol
m=83,7 mg

HOBt m=61 mg

EDC·HCl m=179,22 mg

TEA V=290 µl

Product was purified on a silica gel column in the solvent system CH₂Cl₂:MeOH:NH₄OH
6:1:0,1.

m(OMY)=15,8 mg 95,17% according to UV MS(MH⁺) 1152,53

Performed by: Oresta Makaruha
Witnessed by: Linda Tomašković

Date: November 20, 2000
Date: November 27, 2000

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

VIŠNJA POLJAK

1131

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



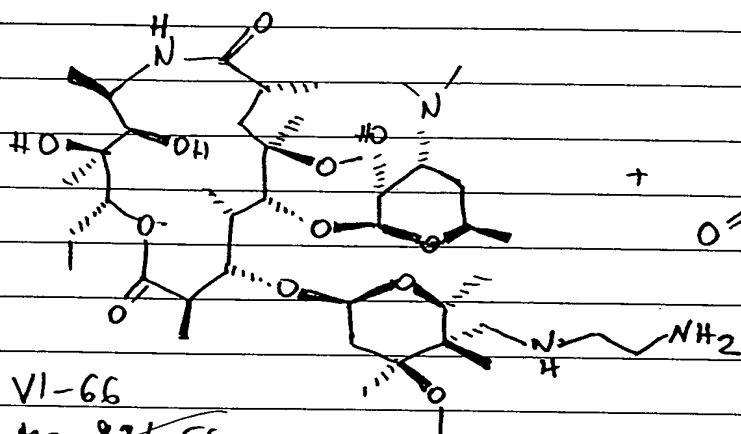
Naslov: _____

Oznaka VI-70

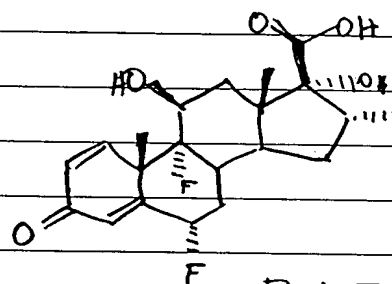
PL 27617

Datum (i): 17.12.2001.

REAKCIJA:

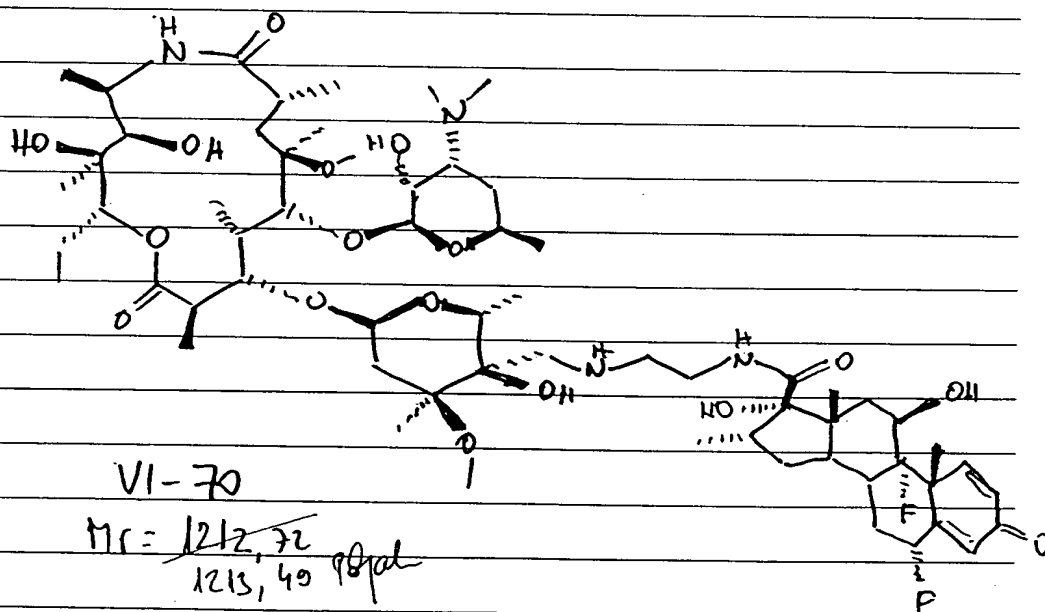


Mr = 834,51
835,08 98%al



Mr = 396,17

TEA
HBT, EDC-Her



Mr = 1212,72
1213,40 98%al

REFERENCA:

Rad obavio: Vikija Rigel

Datum: 17.12.2001.

Rad posvjedočio: Linda Tomášková

Datum: 19.12.2001.

Istraživanje i razvoj

Naslov: _____ Oznaka VI-70

Datum (i): 17.12.2001.

POLAZNI MATERIJAL:

VI-66, $M_r = 834,56$ $m = 0,1411$ g $n = 0,1178$ mmol
 DIKLOMETAN, ~~osušeni~~ ~~izvad~~ molekulska maza 0,4 μm $V = 6,5$ ml
 FLUMETAZON KISELINA, $m = 46,68$ mg $n = 0,1178$ mmol
 TRIETILAMIN, ~~osušeni~~ ~~izvad~~ KOH $V = 0,128$ ml $n = 0,9209$ mmol
 HIDROKSIBENZOTRIAZOL, ACROS $m = 0,03175$ g $n = 0,2356$ mmol
 EDC-HCl $m = 0,4693$ g $n = 0,4012$ mmol

POSTUPAK DOBIVANJA:

postupak dobivanja kao za VI-54 na str. 20

DOBIVENO PRODUKTA:

Nakon otušavanja na hladnoj površini mlakom i sustavu
 otapala CH_2Cl_2 : MeOH: $NH_4OH = 6:1:0,1$

$m(VI-70) = 0,082$ g

$Ms(MH^+) = 607,44$ ~~spektar~~ ~~i~~ ~~analiza~~

Rad obavio: Vesna Poljak

Datum: 17.12.2001.

Rad posvjedočio: Simone Tanasini

Datum: 19.12.2001.

Title: _____

Lab diary ID VI-70
PL 27617

Date (i): 17.12.2001.

REACTION SCHEME:

Scheme in lab notebook No. 1131

Reference:

Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 17.12.2001.
Date: 19.12. 2001.

Title: _____

Lab diary ID VI-70

Date (i): 17.12. 2001.

STARTING MATERIAL:

VI-66 $M_r=834,56$ $m=0,1411$ g $n=0,1178$ mmol
Dichloromethane, dried over molecular sieves 0,4 nm $V=6,5$ ml
Flumethasone acid, $m=46,68$ mg, $n=0,1178$ mmol
Triethylamine, dried over KOH $V=0,128$ ml $n=0,9209$ mmol
Hydroxybenzotriazole, Acros $m=0,03175$ g $n=0,2356$ mmol
EDC \times HC $m=0,1493$ $n=0,4712$ mmol

PREPARATION METHOD:

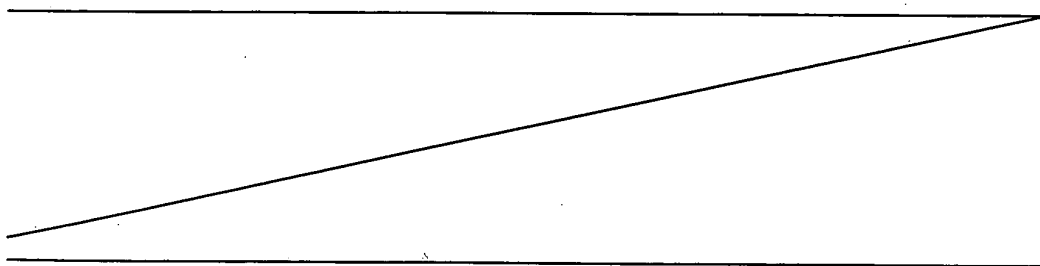
Preparation method as for VI 54 on page 20.

PRODUCT OBTAINED:

After purification on a silicagel column in the solvent system
 $\text{CH}_3\text{Cl}:\text{MeOH}:\text{NH}_4\text{OH}=6:1:0,1$.

$m(\text{VI-70})=0,082$ g HPLC-MS spectra are attached.

$\text{MS}(\text{MH}^+)/2=607,44$ spectras attached



Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 17.12. 2001.
Date: 19.12.2001.

PLIVA d.d.

Istraživanje i razvoj

LABORATORIJSKI DNEVNIK

VIŠNJA POLJAK

1131

Prilaz baruna Filipovića 25, 10000 Zagreb, Hrvatska



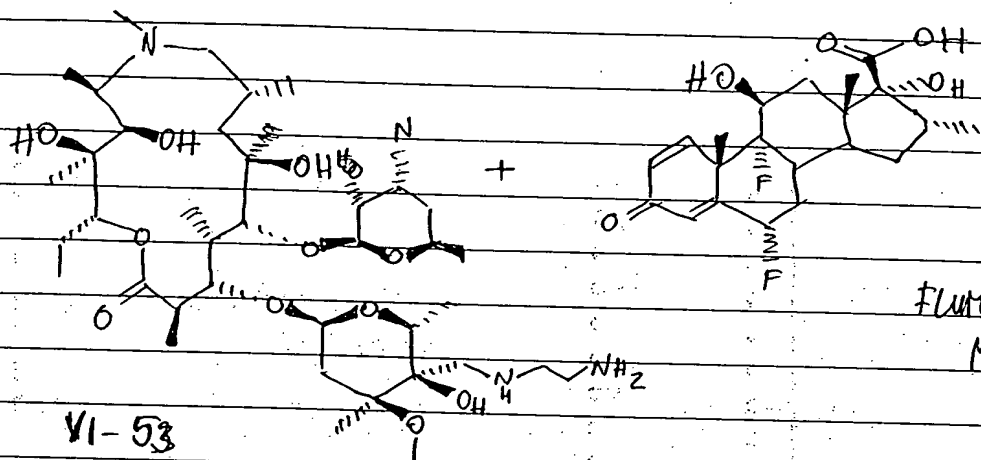
Naslov: _____

Oznaka VI-59

PL 27468

Datum (i): 27. 11. 2001.

REAKCIJA:



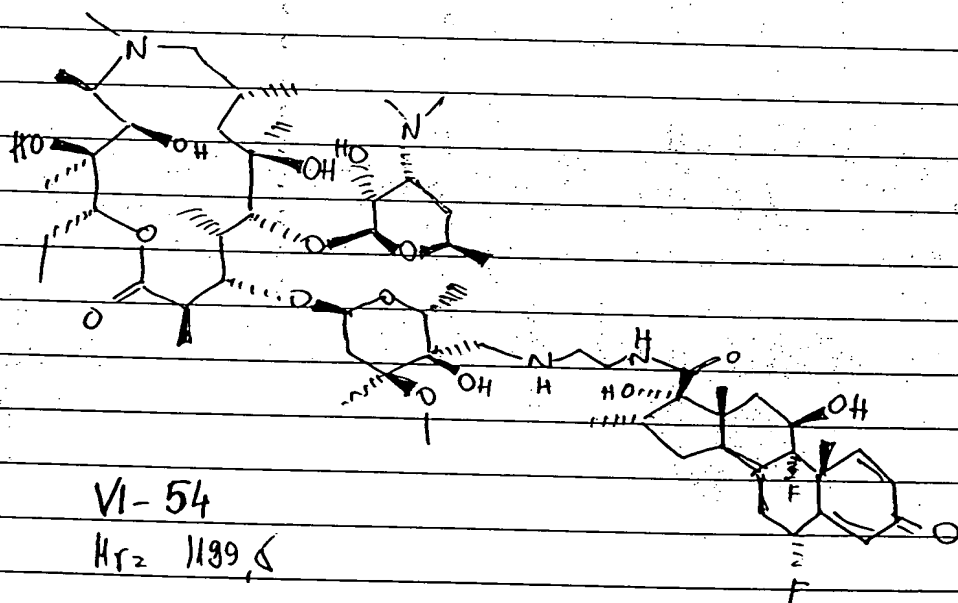
FLUMETASON KISELINA

Mr= 396,42

VI-53

Mr= 820,68

TEA
HOBT, EDC HCl



VI-54

Mr= 1199,6

Zapiska:

Rad obavio:

1/18/2001 Poljak

Datum:

27. 11. 2001.

Rad posvjedočio:

Andre Tomasić

Datum:

29. 11. 2001.

Istraživanje i razvoj

Naslov: _____

Oznaka

VI-54

Datum (i): 27.11.2001.

POLAZNI MATERIJAL:VI-53 ($M_r = 820,58$)FLUTETAZON KISELINA $M_r = 396,42$ TRIETILAMIN, OSUŠEN IBNAD K₂H

HIDROKSIBENZOTRAZOL, ACROS, lot. Nr: A012582601

EDC.HCl

DIKLORMETAN, osušeni Anad molekulske sita 0,4 um

POSTUPAK DOBIVANJA:

U 10 ml diklormetana kroz koji je propuhao argon dodano je 0,1163 g (0,2933 mmol) flutetazonske kiseline. U reakcijsku smjesu dodano je zatim 0,320 ml TEA (2,293 mmol), pri čemu se odmah razbistri. Dodano je zatim 0,0793 g ^{195865 um-2011} HOBt; zatim brzo 0,2408 g VI-53 (0,2933 mmol), te 0,2017 g (1,1732 mmol) EDC.HCl.

Reakcijska smjesa je miješana 24 h na sobnoj temperaturi pod propuhom. Otapalo je zatim upareno, a spoj otisnu na koloni su sustavno otapala CH_2Cl_2 : MeOH: $\text{NH}_4\text{OH} = 6:1:0,1$. Spoj je bio potreban čistiji još jednom (u istom sustavu).

DOBIVENO PRODUKTA: $m(\text{VI-54}) = 0,2141 \text{ g}$ Spektri IR i ¹H-NMR priloženi. $M_s(MH^+) = 1203,0$

Rad obavio:

Vesna Rajak

Datum: 27.11.2001.

Rad posvjedočio:

Ljiljana Tanasinić

Datum: 28.11.2001.

Title: _____

Lab diary ID

VI-54
PL 27468

Date (i): 27.11.2001.

REACTION SCHEME:

Scheme in lab notebook No. 1131

Reference:

Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 27.11. 2001.
Date: 29.11. 2001.

Title: _____

Lab diary ID VI-54

Date (i): 27.11. 2001.

STARTING MATERIAL:

VI-53 (Mr=820,58)
Flumethasone acid, Mr=396,42
Triethylamine, dried over KOH
Hydroxybenzotriazole, Acros, Lot.No. AO12582601
EDC_xHC
Dichloromethane, dried over molecular sieves 0,4 nm

PREPARATION METHOD:

To 10 ml dicloromethane blown up with argon 0,1163 g (0,2933 mmol) of flumethasone acid was added. To the reaction mixture 0,320 ml TEA (2,293 mmol) was then added and the mixture cleared. 0,0793 g (0,5866 mmol) HOBt was then added and then quickly 0,2408 g VI-53 (0,2933) and 0,2017 g (1,1732 mmol) EDC_xHCl.

Reaction mixture was stirred for 24 h at room temperature under argon. Solvent was then evaporated, and the compound purified on a column in the solvent system CH₂Cl:MeOH:NH₄OH=6:1:0,1

The compound had to be purified once again (in the same system).

PRODUCT OBTAINED:

m(VI-54)= 0,2141 g

HPLC-MS spectra are attached.

MS(MH⁺)=1200,0

Performed by: Visnja Poljak
Witnessed by: Linda Tomaskovic

Date: 27.11. 2001.
Date: 29.11.2001.

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